PB87-100582

Characterization of Chemical Waste Site Contamination and Determination of Its Extent Using Bioassays

Battelle Pacific Northwest Labs., Richland, WA

Prepared for

Corvallis Environmental Research Lab., OR

1986

US Department of Commerce
National Technical Information Service

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO. EPA/600/J-86/130	2.
3. PB87-100582	
4. TITLE AND SUBTITLE Characterization of Chemical Waste Site Contamination and Determination of Its Extent Using Bioassays (Journal Version)	5. REPORT DATE 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J.M.Thomas, J.R.Skalski, J.F.Cline, M.C.McShane, W.E. Miller, S.A.Peterson, C.A.Callahan and J.C.Greene	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Battelle, Pacific NW Lab, Richland, WA and US EPA, ERL, Corvallis, OR	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S.Environmental Protection Agency Corvallis, OR 97333	13. TYPE OF REPORT AND PERIOD COVERED Major Publication
	14. SPONSORING AGENCY CODE EPA 600/02

15. SUPPLEMENTARY NOTES

REF: Environmental Toxicology & Chemistry, 5:487-501, June 1986

16. ABSTRACT

Bioassays were used in a three-phase research project to a) assess the comparative sensitivity of test organisms to known classes of chemicals, b) determine if the chemical components in field soil and water samples of unknown chemical composition could be inferred from laboratory studies using pure chemicals and c) investigate kriging (a relatively new statistical "mapping" technique) and bioassays as methods to define the areal extent of chemical contamination.

The algal assay generally was most sensitive to samples of pure chemicals, soil elutriates and water from eight sites with known chemical contamination. Bioassays of nine samples of unknown chemical composition from the Rocky Mountain Arsenal site showed that a lettuce seed soil contact phytoassay was most sensitive. Preliminary evidence suggests that bioassays might be a useful tool in broadly identifying classes of toxic components of contaminated soil. Nearly pure formulations of insecticides and herbicides were less toxic than were their counterpart commercial formulations. This finding indicates that chemical analysis alone may fail to correctly rate the severity of possible environmental toxicity. Finally, we demonstrate that the lettuce seed phytoassay and kriging techniques can be used to map contamination in a portion of the Rocky Mountain Arsenal site and aid in cleanup decisions.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 16
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA/600/J-86/130
JOURNAL ARTICLE

Environmental Toxicology and Chemistry Vol. 5, pp. 487–501, 1986
Printed in the USA. Pergamon Press Ltd.

0730-7268/86 $3.00 + .00
Copyright © 1986 SETAC

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

CHARACTERIZATION OF CHEMICAL WASTE SITE CONTAMINATION AND DETERMINATION OF ITS EXTENT USING BIOASSAYS

J. M. THOMAS,* J. R. SKALSKI, J. F. CLINE, M. C. MCSHANE and J. C. SIMPSON
Battelle, Pacific Northwest Laboratory, Richland, Washington 99352

W. E. MILLER, S. A. PETERSON, C. A. CALLAHAN and J. C. GREENE
U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, Oregon 97330

(*Received* 2 *March* 1985; *Accepted* 27 *November* 1985)

Abstract—Bioassays were used in a three-phase research project to (a) assess the comparative sensitivity of test organisms to known classes of chemicals, (b) determine if the chemical components in field soil and water samples of unknown chemical composition could be inferred from laboratory studies using pure chemicals and (c) investigate kriging (a relatively new statistical "mapping" technique) and bioassays as methods to define the areal extent of chemical contamination.

The algal assay generally was most sensitive to samples of pure chemicals, soil elutriates and water from eight sites with known chemical contamination. Bioassays of nine samples of unknown chemical composition from the Rocky Mountain Arsenal site showed that a lettuce seed soil contact phytoassay was most sensitive. Preliminary evidence suggests that bioassays might be a useful tool in broadly identifying classes of toxic components of contaminated soil. Nearly pure formulations of insecticides and herbicides were less toxic than were their counterpart commercial formulations. This finding indicates that chemical analysis alone may fail to correctly rate the severity of possible environmental toxicity. Finally, we demonstrate that the lettuce seed phytoassay and kriging techniques can be used to map contamination in a portion of the Rocky Mountain Arsenal site and aid in cleanup decisions.

Keywords—Bioassay Chemical wastes Kriging Cleanup Areal dispersion

INTRODUCTION

The purpose of using bioassays to evaluate soils, soil elutriates, and surface and subsurface water from hazardous chemical waste sites is to provide a more direct, integrated estimate of environmental toxicity. Based on bioassay data, chemical waste sites can be ranked according to their toxic potential or mapped for cleanup operations. Since chemical waste sites are seldom contaminated with single compounds, chemical analysis can be expensive and, in fact, uninformative regarding environmental hazard. Two bioassays, *Salmonella typhimurium* (Ames) and *Panagrellus redivivus* (a nematode), have been used successfully in concert with chemical fractionation to identify toxic compounds in cores of lake sediments by Samoiloff et al. [1] They found that the "most toxic fractions contained none of the priority toxic chemicals." Moreover, several different bioassays may be needed to assess the environmental risk of water samples [2].

The objectives of this study were to (a) assess the comparative sensitivity of test organisms to known chemicals, (b) determine if the chemical components in field soil and water samples of unknown composition could be inferred from laboratory studies using pure chemicals and (c) investigate kriging (a relatively new statistical "mapping" technique) of bioassay results as a method to define the areal extent of contamination. In support of these objectives, data are presented on the response of the organisms listed in the Hazardous Materials Assessment Team (HMAT) test protocol [3] to pure chemicals from three chemical subgroups (heavy metals, insecticides and herbicides).

*To whom correspondence may be addressed.

Although the research described in this article was funded wholly or in part by the United States Environmental Protection Agency (EPA), it was not subjected to EPA review and therefore does not necessarily reflect the views of EPA; no official endorsement should be inferred.

The responses of these organisms to actual waste site soils, soil elutriates and water (for which the major contaminants are known) are then evaluated to determine if specific chemical components are probably present (based upon bioassay results). A similar approach is utilized to evaluate Rocky Mountain Arsenal (RMA; Commerce City, CO) waste site soils and wastewater basins for which the chemical constituents must be inferred from past plant operations. Finally, the results of a field study at RMA are presented to demonstrate that bioassay results can be used to prepare a map of suspected contamination that could be useful in cleanup decisions.

METHODS

All pure chemical, waste site soil, surface water and ground water samples were assayed using algae, *Daphnia*, earthworm and root elongation tests in accord with the procedures described in the HMAT protocol for bioassessment of hazardous waste sites [3]. The modified Neubauer seed germination phytoassay [4] was also used to test samples from the Rocky Mountain Arsenal (RMA) and seven other hazardous waste sites. Microtox tests were conducted as outlined in the instrument operation manual [5], and dissolved oxygen depletion rate was determined using the method of Bauer et al. [6].

Heavy metals (copper, cadmium and zinc) were prepared from ACS reagent-grade chloride salts dissolved in deionized water. Water-soluble fractions of aldrin, dieldrin and endrin were made from reagents supplied by Chem Service (Westchester, PA) and the U.S. Environmental Protection Agency (EPA) Repository for Toxic and Hazardous Materials. Aldrin (6-152), dieldrin (6-115a) and endrin (5-171), supplied by Chem Service, were 99% pure, and the reference analytical standards supplied by the EPA repository for aldrin, dieldrin, endrin, chlordane and heptachlor were certified to be more than 97% pure (5,000 ± 250 μg/ml methanol). Dow 2,4-D acid was 94% pure, while Dow Esteron 99® contained 2,4-D at a concentration of 0.485 mg/ml.

Chemicals supplied by the EPA repository were added to deionized water at their water solubilities (as identified by the U.S. EPA [7]). Aldrin, dieldrin and endrin, supplied by Chem Service, were added to deionized water at 10 times their water solubilities, mixed with a magnetic spinbar for 48 h at 24 ± 2°C and filtered through a 0.45 μm membrane filter prior to chemical analysis and bioassessment. Dow Esteron 99 and 2,4-D were assayed at nominal concentrations based on their respective water solubilities [8]. Esteron was assayed as an emulsion in deionized water, while 2,4-D was dissolved in alkaline (pH greater than 10.00 with NaOH) deionized water that was neutralized to pH 7.0 with HCl prior to bioassays. Concentrations in excess of water solubility for aldrin, dieldrin, endrin, 2,4-D and Esteron 99 used in the earthworm and Microtox assays were prepared using Spectro-grade acetone prior to assay. Control assays were run using solvent blanks. The EC50 response values (LC50 for earthworms) for each of the chemicals investigated were obtained using EPA methods [3].

The field study was conducted at the RMA, a site that had been used for the manufacture of antipersonnel gases, herbicides and insecticides, and as an ordinance testing area. Over the years, myriad organic and inorganic compounds were carried through ditches to a series of interconnecting holding basins for disposal.

A waste trench was chosen for study in Basin A (Fig. 1). Four parallel transects (15 m apart) were established on the west side of the basin, each beginning on the north bank of the trench and running south for 90 m (Fig. 2). A logarithmic scale was used beyond the south trench edge to locate five of the sample points on each transect (36 cores, 60 total samples; see below) to account for the possibility that contamination might have moved by some physical means (e.g., wind or water). The first three sample points of each transect fell within the trench, and the fourth was on the top of the south bank. Samples 5 through 9 were located south of the north trench edge at 15, 20, 30, 50 and 90 m, respectively (Fig. 2).

At most sampling points, a split-spoon corer mounted on a hydraulic drill rig was used to take two soil cores, one from the 0 to 15 cm depth and a second from the 15 to 30 cm depth (at locations 4 to 9 in each transect; Fig. 2). Each core was 7.5 cm in diameter and weighed approximately 2 kg. Between sampling points, the split spoon and drill bit were decontaminated by washing them with methanol and rinsing with distilled water. All samples were put in plastic bags, sealed and labeled.

The first two points in each transect were in the trench, which was very wet and soft; thus, two surface samples (to 15 cm deep) were taken with a hand trowel. Sample points L3, N2 and P3 were just over the south bank of the trench and could not be reached, so the split spoon was hammered into the ground and extracted by hand. Because of

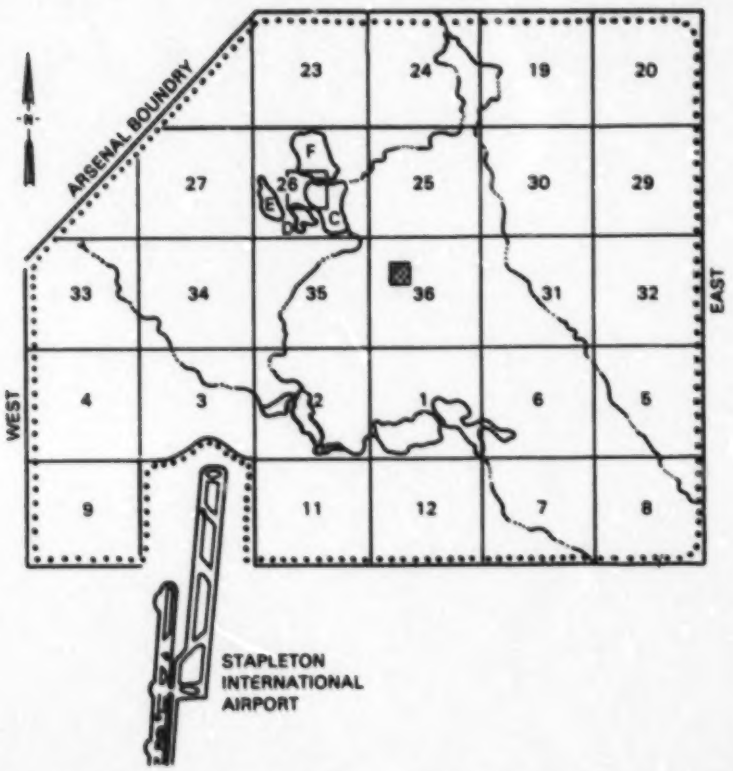

Fig. 1. Location of the study site in Basin A (section 36) at the Rocky Mountain Arsenal. The areas in section 26 labeled C, D, E and F are or were waste ponds.

wetness, only a 0 to 15 cm sample was obtained. A surface sample of undefined depth was taken from M3 because the entire profile was very wet. All samples were air-dried and sieved through a 0.25-in screen.

RESULTS AND DISCUSSION

Pure chemical bioassays

Average EC50 chemical concentrations and respective coefficients of variation (C.V.), based on means, from replicate experiments conducted with each test organism and chemical in a subgroup are reported in Tables 1 to 3. The detailed data in Tables 1 to 3 are presented as ranges in Table 4 to facilitate a comparison of species response for all chemicals in each of the three subgroups, i.e., the range of both parameters (mean and C.V.) for the heavy metal (copper, cadmium, zinc), herbicide (2,4-D, Esteron 99) and insecticide (aldrin, dieldrin, endrin, chlordane, heptachlor) chemical subgroups.

The EC50 or LC50 values obtained with each test organism for copper, cadmium and zinc are similar to those found by other investigators (Table 1). The data in Table 1 also show the algae and *Daphnia* assays to be the most sensitive to copper, cadmium and zinc, followed (in decreasing sensitivity) by the Microtox, dissolved oxygen depletion rate, root elongation and earthworm bioassays.

Two formulations of the same herbicide, 2,4-D acid and Esteron 99 (44.9% 2,4-D), were used in bioassays. All seed species, except wheat, used in the root elongation assay (Table 2) exhibited EC50s of less than 0.1 mg/L 2,4-D. Algae were about 500 times less sensitive to 2,4-D. This is most likely due to the fact that 2,4-D is an auxin analog, which stimulates destructive growth in

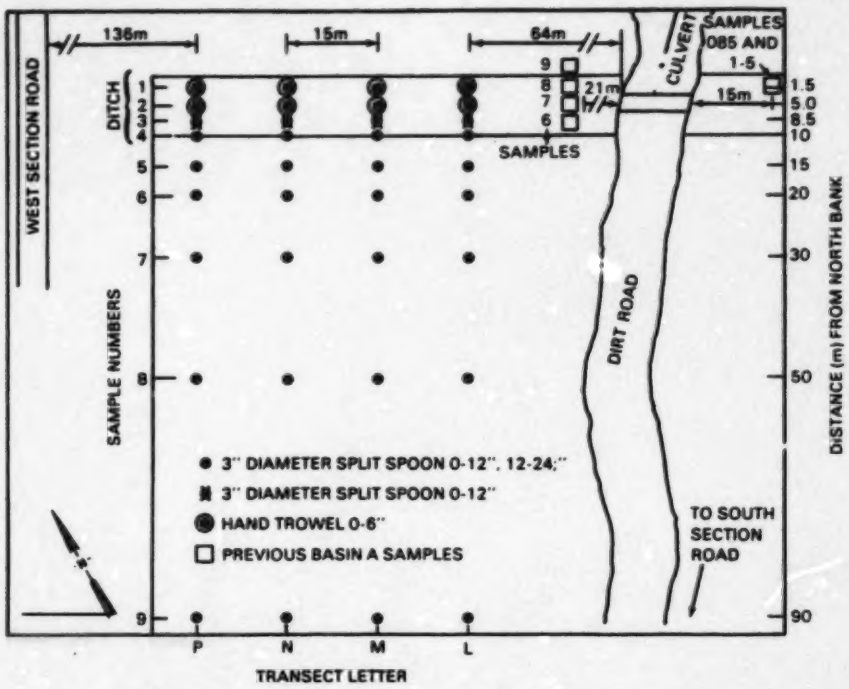

Fig. 2. Location of soil sampling points in Basin A (section 36) at the Rocky Mountain Arsenal.

higher plants in which growth is auxin regulated. Algae do not have auxin growth regulators, but they do react to inhibition of the photosynthesis energy transport process. Thus, as might be expected, the effects of 2,4-D on higher plants and algae differ markedly.

In our experiments, 2,4-D severely affected root elongation, whereas the algae, Microtox and *Daphnia* assays resulted in much higher EC50 values (Table 2). Earthworms tolerated the highest 2,4-D concentrations, a response similar to their response to heavy metals (Table 1). This rank order of sensitivity to 2,4-D is quite different from that observed with heavy metals, for which the root elongation assay resulted in the second highest tolerance.

Comparison of the response of the test organisms to Esteron 99 (a commercial formulation containing 44.9% 2,4-D and undisclosed "inert" ingredients) and the response obtained using 94% pure 2,4-D acid (Table 2) shows that Esteron 99 is significantly more toxic in the algae, *Daphnia* and Microtox assays. There was very little difference in root elongation response for the five plant species tested. *Daphnia* and algae EC50 values were similar for Esteron 99, but *Daphnia* and algae were 18 and 7.4 times, respectively, more sensitive to Esteron 99 than to 2,4-D acid (Table 2). The higher toxic response for Esteron 99 suggests that the inert components in the formulation are also active toxicants, or that the sample preparation process somehow affected bioavailability.

Toxic responses to both water-soluble formulations of aldrin, dieldrin and endrin were only evident for algae and *Daphnia* (Table 3). The algae and *Daphnia* EC50 values for assays using EPA repository aldrin and endrin (Table 3) were higher than the EC50 values for the same insecticides supplied by Chem Service, even though the EPA formulations produced higher concentrations of the active ingredient. The active ingredients are present in higher concentrations in the EPA repository formulations (Table 5) because the EPA methanol formulation was more soluble in water than was

Table 1. Mean EC50 (mg/L), coefficient of variation and number of bioassays for several species using copper, cadmium and zinc

	Copper				Cadmium				Zinc			
Test	$\bar{x}$	C.V.	*n*	Literature value	$\bar{x}$	C.V.	*n*	Literature value	$\bar{x}$	C.V.	*n*	Literature value
Algae	0.043	9	4	0.034 [12]	0.041	15	5	0.050 [13]	0.066	12	5	0.100 [13]
Daphnia	0.064	10	4	0.030 [14]	0.041	20	5	0.047 [14]	0.540[a]	30	5	0.66 [15]
Dissolved oxygen												
0 h	5.4	35	4						5.1		1	
3 h	3.5		1	5.0 [6]					1.5		1	
22 h	7.8	13	2						39.7		1	
Microtox												
5 min	1.98	49	20	1.80 [16]	106	40	5	154 [16]	12.0	50	5	56.0 [16]
15 min	0.3	43	24	0.28 [16]	25	35	5	41 [16]	1.6	45	5	6.1 [16]
30 min	0.3	30	25	0.13 [16]	14	25	5	15 [16]	0.7	35	5	1.4 [16]
Root elongation												
Wheat	24	25	3		66	16	3	76 [17]	334	17	3	
Lettuce	22	20	3		58	15	3	61 [17]	31	10	3	
Radish	55	35	3		58	30	3	128 [17]	53	35	3	
Red clover	45	15	3		57	20	3	27 [17]	43	20	3	
Cucumber	47	25	3		65	35	3	102 [17]	61	30	3	
Earthworm (H_2O)[b]	168	19	3	376[c]	997	2	3	1,440[c]	707	41	3	960[c]
Earthworm (soil)[b]	644		1		1,834		1		628		1	

[a]Value obtained at 72 h; no effect was observed at 48 h.
[b]LC50 values (i.e., the concentration at which 50% mortality occurs).
[c]E.F. Neuhauser, Cornell University, personal communication, 1983.

Table 2. Mean bioassay EC50 (mg/L), coefficient of variation and number of bioassays for two herbicides

	2,4-D			Esteron 99		
Test	$\bar{x}$	C.V.	*n*	$\bar{x}$	C.V.	*n*
Algae	95.8	50	3	12.9	20	3
Daphnia	>240	40	3	13.1	25	3
Dissolved oxygen						
0 h	—			—		
3 h	—			—		
22 h	—			—		
Earthworm (H_2O)	NE			NE		
Earthworm (acetone)[a]	9,830		1	12,800	8	3
Microtox						
5 min	112			10.3		
15 min	107			7.7		
30 min	128			8.4		
Root elongation						
Wheat	0.7	<10	3	0.2	<10	3
Lettuce	<0.1	<10	3	<0.1	<10	3
Radish	<0.1	<10	3	<0.1	<10	3
Red clover	<0.1	<10	3	<0.1	<10	3
Cucumber	<0.1	<10	3	<0.1	<10	3

NE, no biologically important effects.
[a]Reported as LC50. Acetone was used to increase solubility.

Table 3. Mean EC50 values (mg/L) for water-soluble concentrations of aldrin, dieldrin, endrin, chlordane and heptachlor from Chem Service (Westchester, PA) and the EPA Repository of Toxic and Hazardous Materials

Test[a]	Aldrin (Chem Ser./EPA)	Dieldrin (Chem Ser./EPA)	Endrin (Chem Ser./EPA)	Chlordane[b]	Heptachlor[b]
Algae[c]	0.025/0.100	0.055/0.071	0.048/0.114	0.156	0.194[d]
Daphnia	0.025/0.094[e]	0.079/0.107[e]	0.051/0.112[f]	0.165[g]	0.162[h]
Dissolved oxygen					
0 h	—/0.079	NE/NE	NE/NE	0.113	0.129
3 h	—/NE	NE/NE	NE/NE	NE	NE
22 h	—/NE	NE/NE	NE/NE	NE	NE

Miller, W.E., S.A. Peterson, J.C. Greene, and C.A. Callahan. 1985. *J. Environ. Qual.* 14:572 (Table 4). Reprinted with permission [23].

[a]No biologically meaningful effects (NE) were observed for earthworm, Microtox or root elongation assays. Single values resulted from EPA repository samples.

[b]Samples of chlordane and heptachlor were not available from Chem Service.

[c]$n = 3$ for all *Daphnia* and algae bioassays. See Table 4 for the range of coefficients of variation.

[d]Literature value for heptachlor is 0.40 [18].

[e]Literature values for aldrin and dieldrin are 0.028 and 0.190, respectively [19].

[f]Literature value for endrin is 0.352 [20].

[g]Literature value for chlordane is 0.058 [21].

[h]Literature value for heptachlor is 0.078 [22].

Table 4. Summary of EC50 (mg/L) results from Tables 1 to 3, for heavy metals, herbicides and insecticides for six bioassays

	Heavy metals		Herbicides		Insecticides[a]	
Test	Mean	C.V. range	Mean	C.V. range	Mean	C.V. range
Algae	0.04–0.07	9–15	13–96	20–50	0.07–0.19	10–30
Daphnia	0.04–0.54	10–30	13–>240	25–40	0.09–0.17	10–40
Dissolved oxygen rate (3 h)	1.5–3.5		—			
Microtox (30 min)	0.3–106	25–35	8–128			
Root elongation	22–334	10–35	<0.1–0.7	<1–10		
Earthworm (H_2O)[b]	168–997	2–41	—			
Earthworm (acetone)[b]			9,830–12,800	(—)–8	15,000–16,000[c]	

[a]Based on chemicals obtained from the EPA Repository for Toxic and Hazardous Materials.

[b]LC50 values (i.e., the concentration at which 50% mortality occurs).

[c]Erratic results (not listed in Table 3) where water-soluble studies are reported.

the Chem Service powder. Only slightly higher EC50 values were found for dieldrin. Methanol added to solutions (0.005% methanol, v/v) of algal assay medium and *Daphnia* test water (200 mg/L hardness) did not adversely affect either test organism at the maximum methanol concentrations in water-soluble assay medium when EPA repository insecticide samples were assayed.

Our results indicate that algae might be the most broadly sensitive test organism for assays using soil elutriates and water samples, since they responded to water solutions of the major chemical subgroups we studied. A larger group of chemicals must be tested to substantiate this finding. In addition, our results indicate that a potential problem exists if toxic effects are predicted for pure chemicals based on results using commercial formulations (or vice versa). Based on its 2,4-D content, the Esteron 2,4-D preparation should have had minimal effects on aquatic organisms, but the bioassay indicated that the commercial formulation may be more toxic than its active ingredient. Moreover, the lower EC50s obtained using the Chem Service aldrin and endrin suggest the presence of a toxic constituent either not present or not bioactive in the EPA chemical reference standards.

Table 5. Concentrations of aldrin, dieldrin, endrin, chlordane and heptachlor (mg/L) in water-soluble assay samples

Constituent	Source	
	Chem Service	EPA repository
Aldrin	0.025	0.125
Dieldrin	0.096	0.130
Endrin	0.069	0.142
Chlordane	—	0.206
Heptachlor	—	0.197

Miller, W.E., S.A. Peterson, J.C. Greene, and C.A. Callahan. 1985. *J. Environ. Qual.* 14:572 (Table 5). Reprinted with permission [23].
Stock samples supplied by Chem Service and the EPA Repository of Toxic and Hazardous Materials.

Waste site bioassays

Bioassays of soil elutriates from site 085 at RMA (Fig. 2 shows sample location; Fig. 1 shows RMA location) demonstrated that the algal assay was approximately 10-fold more sensitive than the *Daphnia* assay (Table 6). No response was observed using Microtox and lettuce root elongation assays, whereas the earthworm soil test resulted in an LC50 value of more than 25% soil. Such results are typical of those for low levels of heavy metals (Table 1), although other chemicals might be responsible.

In contrast, soil sample 092 (from the Basin A lime pit, section 36; Fig. 1) showed a different and increased toxicity pattern in *Daphnia*, Microtox, root elongation and earthworm assays, which might suggest a stronger influence from known organic components (Table 6). Both Basin F water (from a holding basin for toxic wastes) and a sample of Basin F well water (from well number 26008 located in section 26; Fig. 1) were toxic. Basin F water was toxic to all organisms tested, with EC50 values of less than 1.0% dilution; Basin F well water was much less toxic in algae, *Daphnia* and root elongation assays, and was not toxic at all in the Microtox assay. RMA soil elutriates 1 to 6 and 8 and 9 (upper right, Fig. 2) stimulated rather than depressed algal growth (a not unusual finding for diluted effluents). This lack of an algal toxic response appears to be partially corroborated by results from *Daphnia* assays, which show an EC50 of 72% elutriate dilution for samples 1 to 5 (subsamples of a single large sample collected adjacent to 085, but 1 year later) and site 6 (EC50 of 94%). In contrast, lettuce root elongation results show that 100% soil elutriates are toxic, with samples 1 to 5 being most toxic.

The earthworm and modified Neubauer soil contact bioassays for the same RMA samples confirm some of the lettuce root elongation results and, unlike the other soil elutrite tests, show the presence of bioactive toxicants at sites 1 to 8 and

Table 6. EC50 response or percent inhibition caused by chemical contaminants in Rocky Mountain Arsenal soil, soil elutriate, wastewater and ground water samples

Rocky Mountain Arsenal sample number	Major contaminants	Test					
		Algae[a]	*Daphnia*[a]	Microtox[a]	RE[b]	Modified Neubauer	Earthworm
085	Heavy metals, pesticides	8.3	86	NE	NE	—	>25[c]
092	Heavy metals, pesticides	6.4	25	3.0	61	—	<5.0
F basin water	Heavy metals, DIMP, other organics	0.002	0.003	0.006	1.0	0.5[d]	—
F basin well water	DIMP, other organics	27	21	NE	12	—	—
1-5	Unknown	S	72	NE	72[e]	91[e]	62
6	Unknown	S	94	NE	—	100	55
7	Unknown	NE	NE	NE	32	100	<25
8	Unknown	S	NE	NE	19	92	58
9	Unknown	S	NE	NE	26	13	NE

NE, no biologically significant toxicity observed; DIMP, diisopropylmethylphosphonate; S, growth stimulation.
[a]EC50, % elutriate or % water.
[b]RE, lettuce root elongation test; EC50 % elutriate or % water.
[c]Earthworm 14-d soil test LC50 values; % soil.
[d]LD50 value in % F basin water.
[e]72/100 = 72% inhibition of lettuce root elongation in 100% soil elutriate or seed germination in 100% soil (Neubauer test). Neubauer results are the means of three replicates of 40 seeds each.

low toxicity at site 9. Earthworm LC50s ranged from less than 25% to 62% soil for the same samples, but the sample from site 7 was the most toxic.

A comparison of the soil elutriate, and earthworm and lettuce seed soil contact assay results based on RMA samples suggests the following explanations: (a) these soils contained very low levels of water-soluble heavy metal and pesticide contaminants; or (b) organic compounds chelated the soluble heavy metals so that they were not bioactive; or (c) the water-soluble toxic components in these soils were either leached over time or were strongly absorbed to the clay and organic fractions; or (d) the water-soluble toxic components were inactivated during preparation or testing of elutriates.

In contrast to the RMA samples (Table 6), the response of HMAT test organisms to seven other hazardous waste site soils and soil elutriates (for which major contaminants have also been identified; Table 7), shows algae to be the most sensitive test organism to the bioactive components in all samples except those of the Thiokol waste site (sensitivity of *Daphnia* and algae appeared to be equal for the Hollywood site sample). For the Western Processing No. 22 and Big Hohn Houldt sites, *Daphnia* assay results would rate these samples as low in toxicity potential. Toxicity potential has been classified as low, moderate and highly toxic to describe EC50 values of greater than 75%, 20 to 75% and less than 20% elutriate dilution, respectively [3]. However, the algae assay results would be interpreted as giving a highly toxic response based on the same criteria. Microtox assay results confirm the highly toxic nature of the Western Processing No. 22 elutriate, but this assay was insensitive to the samples from the Western Processing No. 1, Sapp Battery and Thiokol sites. The lettuce root elongation test was the least sensitive to soil elutriate samples from these seven waste sites. With the exception of the Thiokol site, the earthworm soil test was usually less sensitive than algae and *Daphnia* elutriate assays, but it generally gave results corresponding to those of the root elongation and Microtox tests.

We previously established that the root elongation, Microtox and earthworm tests are not very sensitive to three heavy metals (Table 1). Based on the assays of Western Processing No. 11 and No. 22 soils, it appears that the earthworm assay may not be affected by phthalates. However, earthworms were the only test organism to respond to the major chemical, diphenylamine, in the Thiokol soil sample. Unlike most of the RMA samples and the Thiokol sample, the other seven waste site soil elutriates apparently contained toxic, water-soluble chemicals. The algal assay was sensitive to these water-soluble components. Based on this assay alone, five of the seven sites would have been deemed highly toxic (EC50 values ranging from 0.2 to 22%), the Sapp Battery sample would be

Table 7. EC50 response (% soil for the earthworm test) to chemical contaminants in hazardous waste site soil and soil elutriate samples

Waste site	Major contaminants	Test				
		Algae	*Daphnia*	Microtox	RE[a]	Earthworm
Westerm Processing						
1	Heavy metals	28	69	NE	NE	NE
11	Heavy metals, solvents, phthalates	1.8	10	29	41	77[b]
17	Heavy metals, phenols, solvents, pesticides	0.2	5.6	2.2	37	55
22	Heavy metals, phthalates, solvents	22	80	11	NE	NE
Hollywood	Pesticides	24	22	>90	NE	>25
Holder Chemical	Pesticides, herbicides	2.1	3.6	18	3.6	70
Big John Houldt	PAH, other organics	5.4	87	28	NE	<10
Sapp Battery	Heavy metals	41	70	NE	NE	NE
Thiokol	Diphenylamine	NE	NE	NE	NE	35
Sharon Steel	Heavy metals, tar, PAH	0.6	30	99	NE	>75

NE, no biologically significant toxicity observed; PAH, polynuclear aromatic hydrocarbons.
[a]RE, Root elongation test.
[b]Earthworm 15-d soil test LC50 values.

Table 8. Comparison of mean percent lettuce seed mortality in 0 to 15 cm (Experiment 1) and 15 to 30 cm (Experiment 2) fractions of Basin A soil[a]

Transect L				Transect M			Transect N			Transect P			Control		
Plot	0–15 cm[b]	15–30 cm[b]	Difference	0–15 cm[b]	15–30 cm[b]	Difference	0–15 cm[b]	15–30 cm[b]	Difference	0–15 cm[b]	15–30 cm[b]	Difference	0–15 cm[b]	15–30 cm[b]	Difference
1	100[c]	100	0	100	100	0	83	79	+4	100	100	0	21	13	+8
2	100	100	0	100	100	0	100	100	0	100	100	0			
3	86	78	+8	91	78	+13	97	98	−1	76	91	−15			
4	10	22	−11	11	83	−72	92	100	−8	15	96	−81			
5	14	12	+2	19	16	+3	13	9	+4	15	66	−51			
6	100	100	0	32	100	−68	100	100	0	97	100	−3			
7	11	36	−25	8	50	−42	98	95	+3	100	100	0			
8	14	20	−6	17	29	−12	7	10	−3	6	100	−94			
9	12	15	−3	14	65	−51	42	52	−10	22	13	+9			

[a]Subsamples of transects P, N, M, and L 1-3 (0–15 and 15–30 cm) and control were from the same source in each experiment.
[b]Soil samples from 0–15 cm and 15–30 cm fractions were run as separate experiments 2 weeks apart.
[c]Mean % of three replicate dishes of 40 seeds each. The average standard deviation for treatments with seeds that germinated was 3.12 seeds for the 15–30 cm fraction and 2.29 seeds for the 0–15 cm fraction. Details of the assay are given in ref. 4.

Table 9. Comparison of lettuce root elongation (RE), lettuce seed mortality (MN), earthworm mortality (EW) and algal inhibition (S) for two fractions of Basin A soils obtained from the Rocky Mountain Arsenal

	Transect L (0–15 cm)				Transect L (15–30 cm)				Transect M (15–30 cm)				Transect N (15–30 cm)				Transect P (15–30 cm)			
Plot	MN[a]	RE[b]	EW[c]	S[d]	MN[a]	RE[b]	EW[c]	S[d]	MN[a]	RE[b]	EW[c]	S[d]	MN[a]	RE[b]	EW[c]	S[d]	MN[a]	RE[b]	EW[c]	S[d]
1	100	53		11	100	53		11	100	58	100	4	83	20	100	17	100	25	100	15
2	100	56	100	39⁻	100	56	100	39	100		100	9	100	31	100	69⁻	100		100	
3	86	42		50⁻	86	42		50⁻	78				97	44		12	91	2	0	24
4	10	21		10	22	0		49⁻	83	14		14	100	4		26	96	10⁺	50	4
5	14	29		9	12	21⁺		25	16	45⁺	0	1	9	35⁺	0*	25	66	23	0	17
6	100	40		25	100	9	0*	27	100	10	100	13	100	8		30	97	15	100	14
7	11	27		20	36	18		23	50	21	0	7	95	30	100	5	100	8	100	8
8	14	11		35	20	14		23	29	33	0	10	10	17		21	100	0	100	19
9	12	20		27	15	33	0*	40	65	20	0		52	15	0	39	13	1	0	30

[a]Percent lettuce seed mortality. Each value is the mean mortality of three replicate plates of 40 seeds, each grown in soil.
[b]Percent of control lettuce root length (based on soil elutriate). Values with superscript plus (+) were greater than controls.
[c]Earthworm percent mortality using 100% soil (one replicate of 10 earthworms). Values with superscript asterisk (*) are questionable since soils had been used previously to prepare elutriates.
[d]EC50 values in % elutriate are denoted by superscript minus (−), while values with no superscript were stimulatory.

moderately toxic and the Thiokol elutriate would be designated nontoxic (the earthworm assay suggests that this sample is moderately toxic). We believe that the results, based on bioassays for these seven waste sites, offer an initial validation of our test protocol premise that a group of diverse bioassays is preferable to any single test.

Bioassays of field study soils

Lettuce seeds were used in the phytoassays of the RMA field study soils since they are more sensitive than other seeds [4] and since our previous work showed these soils to be phytotoxic (Table 6). The mortalities (mean of three subsamples from each core fraction; Table 8) indicate that four samples in transect M and three in transect P showed large differences as a function of depth, suggesting that the contaminants had either migrated below 15 cm or were purposely placed there. We found no records to support the latter argument and conclude that the toxic material had migrated.

The results for all other HMAT bioassays conducted using Basin A field study soils are shown in Table 9. Sample unavailability either precluded analyses or curtailed the number of assays that could be conducted. *Daphnia* or Microtox bioassay results are not included because no response was observed in 10 and 36 assays, respectively.

Results from the algal assay revealed that small quantities of elutriate from all but four samples were stimulatory. The four elutriate samples that inhibited algae were obtained on or very near the waste trench on transects L and N. According to

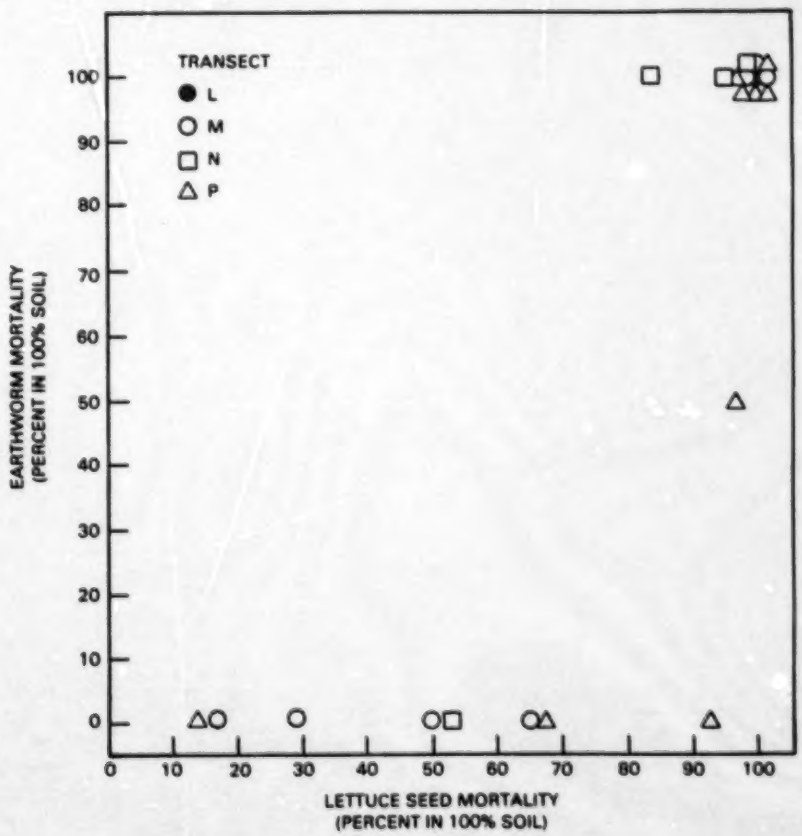

Fig. 3. Comparison of earthworm and lettuce seed mortality using Basin A soils from the Rocky Mountain Arsenal.

the criteria outlined in [3], these sites would be classified as moderately toxic. Interestingly, only 1 to 14% elutriate from transect M samples was needed to stimulate algal growth. Based on the algal bioassay results, we conclude that the toxic components detected using lettuce seeds (Table 8) were probably not due to high levels of any of the water-soluble heavy metals, herbicides or insecticides (except perhaps at sites L2-4 and N2) that we tested, since our results using pure chemicals (Tables 1 to 3) showed depressed algal growth in the presence of these contaminants.

Results of the earthworm bioassay are presented as a function of lettuce seed mortality in Figure 3. Except for plot P3 soil, earthworms were severely affected when placed in Basin A soil samples, where lettuce seed mortalities were greater than 70%. In contrast, the five soil samples that caused 20 to 70% lettuce seed mortality resulted in no earthworm deaths. Thus, for these Basin A samples, it appears either that lettuce seeds are more sensitive to lower levels of a toxic component that does not affect earthworms, or that earthworms are more resistant to the same toxicant.

Results from the lettuce root elongation (based on elutriates) and the lettuce seed mortality (based on intact soil) bioassays are compared in Figure 4. There was only a slight correlation based on sam-

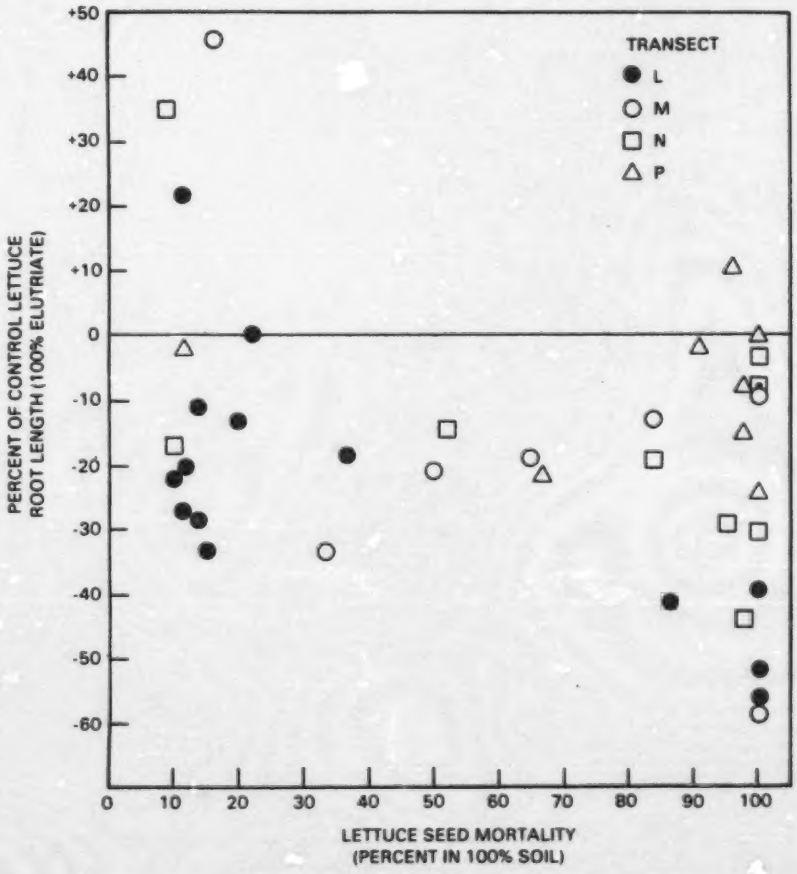

Fig. 4. Comparison of lettuce root elongation and lettuce seed mortality using Basin A soils from the Rocky Mountain Arsenal.

ples from transect L. Thus, it appears that the phytotoxic component that impairs lettuce seed germination may not be water-soluble or does not affect root elongation.

Cleanup decisions based on bioassays and kriging

One way to depict the lettuce seed mortality patterns at each depth (Table 8) is to prepare a contour map based on the observations. We estimated contours for a map of lettuce seed mortality using a relatively new statistical technique called kriging, which was developed for use in the mining industry and is used principally in Europe and South Africa [9,10]. Kriging is a weighted, moving-average technique for calculating point estimates or block averages over a specified grid. The derivation of the kriging weights takes into account the proximity of the observation to the point or area of interest, the structure of the observations (i.e., the relationship of the squared difference between pairs of observations and the intervening distance between them) and any systematic trend or drift in the observations. Additionally, kriging provides a variance estimate that can be used to construct a confidence interval for the true value. Contour maps are prepared from the kriging estimates. Our results (based on block kriging) are presented in Figures 5 and 6; they clearly show that there were lettuce seed mortality differences at the two depths. Estimated contamination is greater at the 15 to 30 cm depth than at the 0 to 15 cm depth. This was also indicated by our qualitative analyses of results (Table 8).

We believe that such maps are useful in site cleanup decisions. As a possible scenario, we selected 30% lettuce mortality as a criterion for cleanup of this Basin A site (heavy line in Figs. 5 and 6). The areas enclosed by the solid lines would be targeted for cleanup. Unfortunately, the cleanup

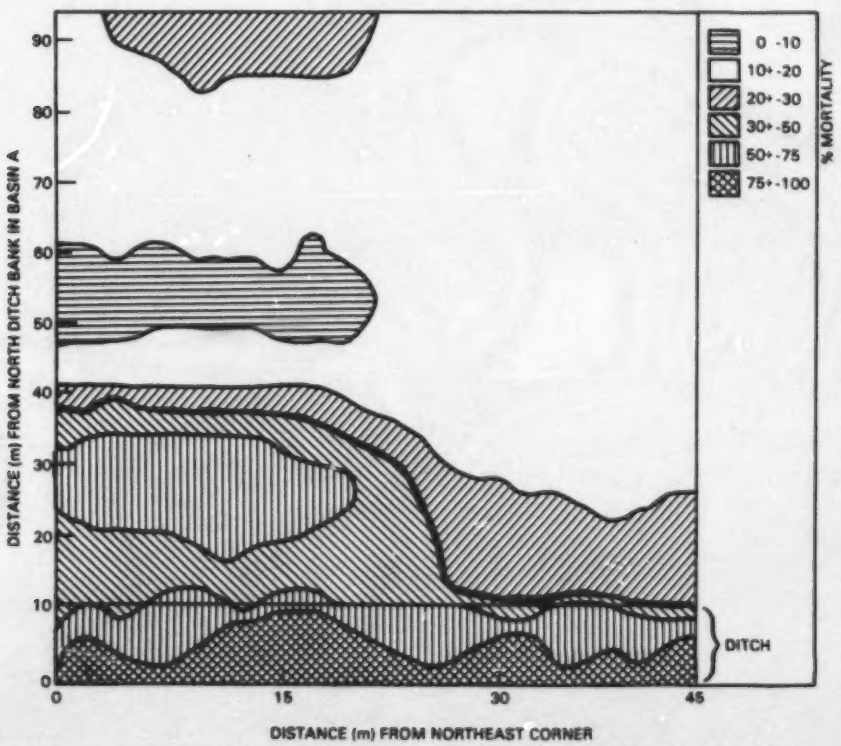

Fig. 5. Estimated lettuce seed mortality (based on kriging) for the 0–15 cm soil fraction from the Rocky Mountain Arsenal.

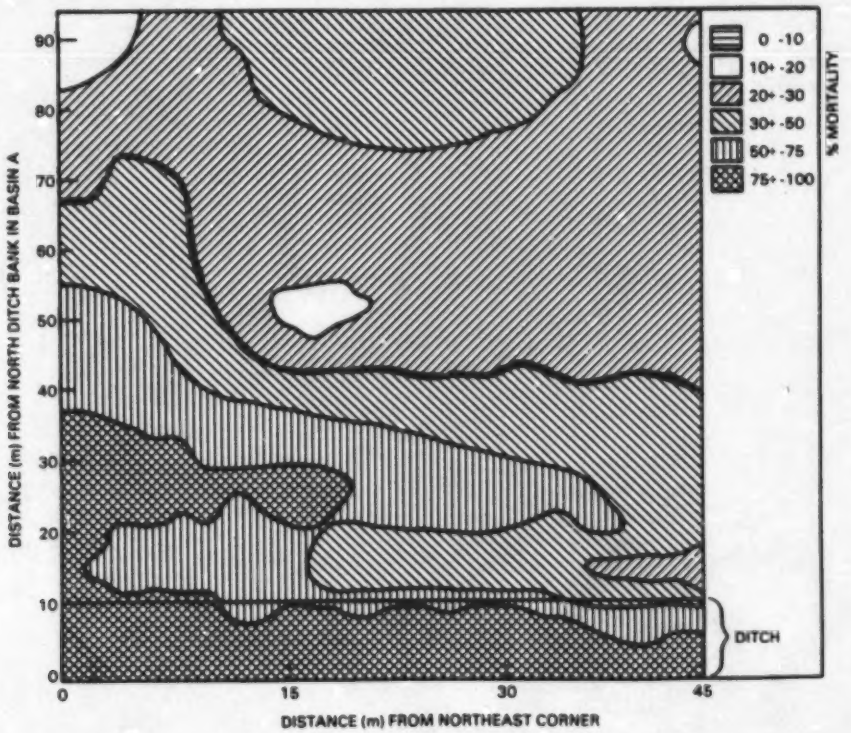

Fig. 6. Estimated lettuce seed mortality (based on kriging) for the 15–30 cm soil fraction from the Rocky Mountain Arsenal.

decision would be different for the 0 to 15 cm (Fig. 5) and the 15 to 30 cm (Fig. 6) fractions. While this difference complicates decision making, the available data (Table 8) and the kriging maps (Figs. 5 and 6) show that the field situation is complex, and that cleanup decisions based solely on either soil fraction would not result in a "clean" site. Cleanup using the 30% mortality contour of the 15 to 30 cm samples would remove all known contamination, but samples were not taken below this depth.

It appears that bioassays of field samples and subsequent kriging analyses offer a practical method to aid in cleanup decisions based on environmental toxicity, especially when accompanied by error estimates for the mortality isopleths. We did not present confidence limits here (limits are dependent on data density, whether block or point kriging is used and the contour of concern; the limits in our study averaged between ±10 and 25%) because of some statistical questions raised by nonphysical mechanism(s) of pollutant spread [11].

SUMMARY

A three-phase research program was conducted to (a) assess the comparative sensitivity of bioassay test organisms to known chemicals, (b) determine if the bioactive chemical subgroups present in field samples could be inferred from bioassay results and (c) map the extent of contamination at an existing hazardous chemical waste site using bioassay results and kriging.

Tests using heavy metals (copper, cadmium and zinc) showed that algae and *Daphnia* bioassays were most sensitive, followed, in order of increasing tolerance, by the Microtox, dissolved oxygen depletion rate, root elongation and earthworm (soil contact) bioassays. Good within-test organism and element test precision was attained, since the

majority of C.V. values were below 25%. Root elongation was most sensitive to 94% 2,4-D acid and a commercial 2,4-D formulation (44.9% pure). Algae, Microtox and *Daphnia* assays exhibited similar 2,4-D tolerances, whereas the earthworm assay was most resistant. A 44.9% 2,4-D formulation was between 7 and 18 times more toxic in the algae, *Daphnia* and Microtox assays than was the nearly pure 2,4-D acid, which indicated that the inert component of the commercial formulation was also toxic. Only algae and *Daphnia* exhibited a toxic response to water-soluble forms of the insecticides aldrin, dieldrin and endrin from EPA repository and commercial sources. As with the two herbicide sources, the commercial form of two of the insecticides was more toxic.

Thus, testing of herbicides and insecticides intended for field use suggests that bioassays may be necessary to assess the bioavailability of components in commercial formulations. Based on assay results from all three chemical subgroups, the algae bioassay appears to be the most sensitive. Since earthworms were most resistant to chemicals from the three subgroups, an additional, more sensitive soil contact phytoassay based on lettuce seeds was used in further studies.

Our evaluation of soils, soil elutriates and surface and ground water samples from the RMA and seven other sites where the major contaminants were known shows that results from bioassays appear to offer promise as useful aids in ascertaining the presence or absence of some types of contamination. Again, algal bioassays were generally most sensitive, but our results indicate it may not be possible to estimate the toxic constituents for all samples in the absence of other bioassay results. Bioassays of nine additional RMA soil samples, for which the contaminant history was unknown, showed that the earthworm and lettuce seed soil contact bioassays were most sensitive, while elutriates generally stimulated algal growth. The lettuce root elongation test, using 100% elutriate, also indicated the presence of toxic components but was less sensitive than the lettuce seed soil contact test. These results suggested the presence of low levels of water-soluble metals and possibly pesticides.

To illustrate how bioassay results could be used to map the toxic potential of a waste site, a field study was conducted at the RMA. Lettuce seed mortality maps were developed by kriging the results from phytoassays. Estimated mortality over the sampling area (as well as observed mortality at sample points) was greatest at the 15 to 30 cm depth. These results indicate that the bioassay of soil samples from a statistically designed field study area, accompanied by kriging, can aid in defining the extent of contamination and in site cleanup decisions. Other bioassays conducted using these same samples (when sufficient sample was available) showed that the modified lettuce seed phytoassay was most sensitive, followed by the earthworm assay. Earthworm mortality was correlated only with lettuce seed mortality of greater than 70%. Thus, the earthworm assay may lack the sensitivity needed for mapping other sites.

Acknowledgement—The technical help of Dr. Dave Thorne, Dr. Bill Trautman and Mr. Brian Anderson, and the cooperation of Dr. William McNeill of the Rocky Mountain Arsenal, were essential in accomplishing this research. At the Pacific Northwest Laboratory, Mary Jo Harris supplied technical support. Gail Poole typed the manuscript. This work was supported by the U.S. EPA Environmental Research Laboratory in Corvallis, Oregon, under related services agreement TD 1598 with the U.S. Department of Energy contract DE-AC06-76RLO 1830. Preliminary research on some of the methodology used in this effort was supported by the Office of Health and Environmental Research, U.S. Department of Energy.

REFERENCES

1. **Samoiloff, M.R., J. Bell, D.A. Birkholz, G.R.B. Webster, E.G. Arnott, R. Pulak** and **F. Madrid.** 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. *Environ. Sci. Technol.* 17:329–333.
2. **Mount, D.I., N.A. Thomas, T.J. Norgerg, M.T. Barbour, T.H. Roush** and **W.F. Brandes.** 1984. Effect of ambient toxicity testing and in stream community response on the Ottawa River, Lima, Ohio. OWEP8401. U.S. Environmental Protection Agency, Permits Division, Washington, DC.
3. **Porcella, D.B.** 1983. Protocol for bioassessment of hazardous waste sites. EPA 600/2-83-054. U.S. Environmental Protection Agency, Corvallis, OR..
4. **Thomas, J.M.** and **J. F. Cline.** 1985. Modification of the Neubauer technique to assess toxicity of hazardous chemicals in soils. *Environ. Toxicol. Chem.* 4:201–207.
5. **Beckman Inc.** 1982. *Microtox System Operating Manual.* Beckman Instruments, Inc., Microbics Operations, Carlsbad, CA.
6. **Bauer, N.J., R.J. Seidler** and **M.C. Knittel.** 1981. A simple, rapid bioassay for detecting effects of pollutants on bacteria. *Bull. Environm. Contam. Toxicol.* 27:577–582.
7. **U.S. Environmental Protection Agency.** 1979. Water-related environmental fate of 129 priority pollutants. EPA 440/4-79-029a and b. Office of Water Planning and Standards, Washington, DC.
8. **Worthing, C.R.,** ed. 1979. *Pesticide Manual,* 6th ed. British Crop Protection Council, London.

9. **Journal, A.G.** and **C.H.H. Huijbregts.** 1978. *Mining Geostatistics.* Academic Press, New York, NY.
10. **Clark, I.** 1982. *Practical Geostatistics.* Applied Science Publishers, London.
11. **Journal, A.G.** 1984. New ways of assessing spatial distributions of pollutants. In G. E. Schweitzer and J. A. Santolucito, eds., *Environmental Sampling for Hazardous Wastes.* American Chemical Society Symposium Series 267. American Chemical Society, Washington, DC, pp. 109–118.
12. **Chiaudani, G.** and **M. Vighi.** 1978. The use of *Selenastrum capricornutum* batch cultures in toxicity studies. *Mitt. Int. Ver. Limnol.* **21**:316–329.
13. **Bartlett, L., F.W. Rabe** and **W.H. Funk.** 1974. Effects of copper, zinc and cadmium on *Selenastrum capricornatum. Water Res.* **8**:179–185.
14. **Canton, J.H.** and **D.M.M. Adema.** 1978. Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments. *Hydrobiology* **59**:135.
15. **U.S. Environmental Protection Agency.** 1980. Ambient water quality criteria for zinc. EPA 440/5-80-079. Office of Water Regulations and Standards Criteria and Standards Division, Washington, DC.
16. **Qureshi Ansar, A., R.N. Coleman** and **J.H. Paran.** 1983. Evaluation and refinement of the Microtox test for use in toxicity screening. *Proceedings*, First International Symposium of Toxicity Testing Using Bacteria, National Water Research Institute, Canada Center for Inland Waters, Burlington, Ontario, Canada.
17. **Ratsch, H.C.** 1983. Interlaboratory root elongation testing of toxic substances on selected plant species. EPA-600/3-83-051. U.S. Environmental Protection Agency, Corvallis, OR.
18. **U.S. Environmental Protection Agency.** 1980. Ambient water quality criteria for heptachlor. EPA 440/5-80-052. Office of Water Regulations and Standards Criteria and Standards Division, Washington, DC.
19. **Johnson, W.W.** and **M.T. Finley.** 1980. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates.* Resource Publication 137. U.S. Department of Interior and Wildlife Service, Washington, DC.
20. **U.S. Environmental Protection Agency.** 1980. Ambient water quality criteria for endrin. EPA 440/5-80-047. Office of Water Regulations and Standards Criteria and Standards Division, Washington, DC.
21. **Randall, W.F., W.H. Dennis** and **M.C. Waener.** 1979. Acute toxicity of dechlorinated DDT, chlordane, and lindane to bluegill *Lepomis macrochirus* and *Daphnia magna.* Bull. Environm. Contam. Toxicol. **21**:849.
22. **Macek, K.J., M.A. Lindberg, S. Sauter, K.S. Buxton** and **P.A. Costa.** 1976. Toxicity of four pesticides to water fleas and fathead minnows. EPA 600/3-76-099. U.S. Environmental Protection Agency, Duluth, MN.
23. **Miller, W.E., S.A. Peterson, J.C. Greene,** and **C.A. Callahan.** 1985. Comparative toxicology of laboratory organisms for assessing hazardous waste sites. *J. Environ. Qual.* **14**:569–574.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.